

pam
QW127
1902
A13e

90200
D. D. BEROLZHEIMER,
317 SOUTH 18TH ST.
PHILADELPHIA.

*Copies
of
Nathaniel Gildersleeve*

THE ETIOLOGICAL SIGNIFICANCE OF THE ACID-
RESISTING GROUP OF BACTERIA, AND THE
EVIDENCE IN FAVOR OF THEIR BOTAN-
ICAL RELATION TO BACILLUS
TUBERCULOSIS.

BY

A. C. ABBOTT, M.D.,

AND

N. GILDERSLEEVE, M.D.

FROM THE

UNIVERSITY OF PENNSYLVANIA MEDICAL BULLETIN,

JUNE, 1902.

WELLCOME INSTITUTE LIBRARY	
Coll.	welMomec
Coll.	pam
No.	QW 127
	1902
	A13e



22200045542

THE ETIOLOGICAL SIGNIFICANCE OF THE ACID-
RESISTING GROUP OF BACTERIA, AND THE
EVIDENCE IN FAVOR OF THEIR BOTAN-
ICAL RELATION TO BACILLUS
TUBERCULOSIS.*

BY A. C. ABBOTT, M.D.,

Professor of Hygiene and Bacteriology, University of Pennsylvania,

AND

N. GILDERSLEEVE, M.D.,

*Assistant in Bacteriology, University of Pennsylvania, and Fellow of
the Rockefeller Institute for Medical Research.*

THE following is the account of an investigation conducted in the Laboratory of Hygiene of the University of Pennsylvania between October, 1901, and June, 1902. The expenses incidental to the work were generously defrayed by the Rockefeller Institute for Medical Research, for which the authors here express their indebtedness.

PREFACE.

For a number of years it has been known that bacteria are occasionally encountered which in morphology and staining reactions are so like bacillus tuberculosis as readily to be mistaken for that organism by microscopic methods of study. Through more or less recent investigations our acquaintance with this group has been greatly extended. We have learned that its members are pretty widely distributed in nature; that most of them are susceptible of artificial cultivation; that a number have the power, when de-

* Presented in abstract at the meeting of the Association of American Physicians, Washington, D. C., April 29 and 30, and May 1, 1902.

posited in the tissues of certain animals, of causing lesions in many ways suggestive of those of genuine tuberculosis; that in the course of their growth in the tissues of animals they frequently develop, as is occasionally true of bacillus tuberculosis, after the fashion of the actinomyces or ray fungi, which speaks for their botanical relationship to bacillus tuberculosis; and that, finally, they are frequently encountered upon the fodder and in the excretions and secretions of many domestic animals, some of which are especially prone to tubercular infection. From the foregoing it is evident that we have to deal with a group of bacteria that simulate the exciting cause of tuberculosis to such an extent as to warrant the opinion that they have not only been the cause of error, but are likely so to continue unless we are more or less intimately acquainted with them.

The repeated detection of the members of this group on the food, in the excretions and secretions, and in the surroundings of domestic animals on which we depend for food, renders them of special interest to the practical hygienist. The frequency with which they have been encountered in butter and in milk, for instance, is at once of significance in suggesting such questions as led to this investigation, viz., May not some, at least, of the published statements concerning the presence of tubercle bacilli in milk and butter be due to the mistaking of these organisms for true tubercle bacilli? When present are they to be regarded as but accidental contaminations from the environment under which such products were collected or manufactured, or is it possible for them to have been present as disease-producing factors in the animals from which the milk originally came?

In view of the foregoing facts and questions suggested by them it was deemed advisable to investigate the pathogenic possibilities of this group not only as exhibited upon the small animals ordinarily used for laboratory experimentation, but also upon the larger domestic animals that are susceptible to tubercular infection; to determine their frequency and distribution in butters of various grades and from various sources, and to decide, in so far as possible, their botanical relationship to bacillus tuberculosis. In a number of aspects this problem is but a repetition of work done by others, but in its bearings upon those domestic animals and

their products that serve as sources of food-supply it was regarded as of sufficient importance to warrant careful review. In so far as we are aware, the phase of our work that is, from the practical stand-point, of greatest importance—notably the etiological significance of this group of bacteria for larger domestic animals—has received but little attention from others interested in the subject, and we believe the results of our studies upon this point justify the belief that the confusing, acid-resisting group of bacteria may be dismissed from consideration as probable factors in the causation of tuberculosis or of any condition indistinguishable from it by the ordinary channels through which animals are likely to be infected.

At the time of its discovery bacillus tuberculosis was believed to differ from all other bacteria, except the bacillus of leprosy, in the peculiarity of its staining reactions. When once stained by appropriate methods it is not, as is well known, readily deprived of its color by the ordinary acid decolorizing solutions; it is designated, therefore, as “acid-resisting” or acid-proof (“*sauerfest*”). On this characteristic, probably more than on any other, has reliance been placed in the identification of this organism by microscopic methods.

Shortly after the discovery of bacillus tuberculosis, however, attention was called by Zahn¹ to the presence in the sputum of a non-tuberculous individual of bacilli having more or less the morphology of bacillus tuberculosis, and, like that organism, having the property of resistance to acid decolorization. After another brief interval Alvarez and Tavel,² Matterstock,³ and Bitter,⁴ announced as a result of their observations that in the normal smegma there were often to be encountered bacilli of like characteristics; and Cramer⁵ and de Giacomi⁶ noted the presence of acid-resisting, pseudotubercle bacilli in the normal intestinal contents of man, so that as means of identification the morphology and staining peculiarities of bacillus tuberculosis soon came to be regarded as not infallible.

With increasing frequency during more recent years there have appeared in medical and bacteriological literature additional references to bacteria that in staining reactions and morphology are so strikingly like bacillus tuberculosis as readily to be mistaken for

it on casual examination by the microscope alone, but which may, in most cases, be easily distinguished by their behavior under artificial cultivation and by their effects upon susceptible animals. In a few cases these organisms have been discovered in conditions not entirely above suspicion in so far as the possibility of their having been of tuberculous nature is concerned, but in many instances they were found under circumstances that might reasonably be regarded as having nothing to do with tuberculosis and as unlikely to have been contaminated with tuberculous matters.

Prominent among the group of contributors to this subject, and the conditions under which their discoveries were made, may be mentioned Fraenkel,⁷ Pappenheim,⁸ and Rabinowitsch,⁹ who noted the occurrence of these confusing organisms in the sputum from cases of pulmonary gangrene; Laabs¹⁰ and Moeller,¹¹ who detected them in the saliva of non-tuberculous individuals; Dietrich,¹² who found them in a suppurating ovarian cyst; Petri,¹³ Rabinowitsch,¹⁴ Hormann and Morgenroth,¹⁵ Grassberger,¹⁶ Herbert,¹⁷ Weissenfeld,¹⁸ Korn,¹⁹ Ascher,²⁰ Coggi,²¹ Herr and Beninde,²² and Tobler,²³ who record their more or less frequent occurrence in butter; Moeller,²⁴ who found them upon certain sorts of fodder and in stable manure; Severin,²⁵ who discovered them in the dung of horses, Capaldi,²⁶ in that of cows, and Ferran,²⁷ in that of cows, horses, and man; and, finally, Cowie,²⁸ who has shown them to be fairly frequent in the smegma about the vulva, mammae, or anus of normal cows, horses, dogs, guinea-pigs, and white rats.

From the foregoing it is evident that the members of this group are widely distributed, and because of the manifest likelihood of their leading to error are worthy of careful consideration.

It is needless at this time to dwell upon the details of the thirty odd organisms of this group that have been found microscopically and isolated in pure culture. It will suffice to say that when studied in pure culture they do not apparently represent more than, at most, two species, the rest being but varieties, possessed of certain fundamental group characteristics. The bacillus of leprosy, probably the most important of the organisms simulating bacillus tuberculosis, cannot with certainty be said to have been cultivated, nor has leprosy or anything like it been reproduced in animals by the inoculation of leprosy tissues. *Bacillus smegmatis*,

probably identical with the so-called bacillus of syphilis, can be cultivated only by special methods, but, so far as we know, is without disease-producing power, though in this respect it has received little attention. The balance of the group, frequently encountered under conditions of saprophytism, may be isolated and grown artificially. In some cases they are isolated readily, in others with difficulty; the reason for the difficulty being, as a rule, that the suspicious bacilli are present with other more rapidly growing species, and are so in the minority that their separation from the other species by culture methods often results in failure. In the case of those occurring in butter but little trouble is usually experienced. When, for instance, butter containing them is injected into the peritoneum of guinea-pigs conditions are created, through the irritating action of the fatty vehicle, that favor their growth. Under these circumstances they are almost uniformly capable of causing pathological lesions, and may with little difficulty be isolated from such lesions in pure culture unless, as occasionally happens, other pathogenic species be at the same time present in the butter injected.

Though resistant to decolorization by acids, this peculiarity is by no means absolute, and the results of our studies upon this phase of the subject lead us to believe that many if not all of the members of the group may be at once distinguished from bacillus tuberculosis by their comparatively low resistance to acid decolorizers. All of them, it is true, retain their color for longer or shorter periods when acted upon by such acid-decolorizing solutions as acetic acid (5 per cent.) and alcohol, hydrochloric acid (3 per cent.) and alcohol, sulphuric acid (5 per cent.) in water; but they lose it almost instantly if treated by the nitric-acid solution (25 to 30 per cent. in water), employed in the original Koch-Ehrlich process for the microscopic identification of bacillus tuberculosis—a fact that in our experience serves at once to distinguish them from genuine tubercle bacilli.*

In morphology the members of this group, whether they be susceptible of artificial cultivation or not, are in certain phases of their development strikingly like bacillus tuberculosis. They are

* See Cowie. The Physician and Surgeon, Detroit and Ann Arbor, January, 1902.

seen as short, slender, usually beaded rods, in some cases being almost identical in form and size to the tubercle bacillus. (See Fig. 1, *a* and *b*.) In a few instances the only point of resemblance is their resistance to decolorization by acids. Occasionally they are clubbed or bulbous, sometimes a little spindle-shaped; but in nearly all cases they are sufficiently suggestive of tubercle bacilli to cause confusion if found microscopically in very small numbers in a specimen of urine, milk, or butter, for instance, especially if the staining method of Gabbet or of some one or another of the comparatively weak acid decolorizers had been employed. In this connection we should not lose sight of the fact that the morphology of true tubercle bacilli is occasionally seen to deviate from that given in the classical descriptions of it, though its staining characteristics remain fairly constant.

When studied under conditions of artificial cultivation those that are capable of growth, which would probably include those most widely distributed, are at once easily distinguished from the exciting factor of tuberculosis. All grow more or less readily at from 20° to 22° C., and all, with the possible exception of those found in coldblooded animals, quite readily at 37° to 38° C. Some grow more rapidly than others, but all much more rapidly than bacillus tuberculosis under similar conditions. For instance, a growth of bacillus tuberculosis under the most favorable conditions is rarely seen earlier than from seven to ten days after transplantation; while with the confusing group growth, even to the extent of distinct colony formation, is commonly seen in from thirty-six to forty-eight hours—sometimes a little earlier, sometimes a little later, but never so delayed as to be confused from this standpoint with the tubercle bacillus.

The character of the growth, too, is quite distinct; but from our studies the difference is more often one of degree than of kind. The growth of bacillus tuberculosis upon artificial media is, as is well known, characterized by the occurrence of coarse, hard, granular masses that can only with difficulty, if at all, be broken up in fluids so as to form a homogeneous suspension; while that of the confusing group begins and continues as a dry, wrinkled, rapidly spreading, lustreless layer that subsequently takes on in some cases a pale or even distinct salmon color, in others a decided

orange hue. If this layer be examined optically and by the sense of touch the hard granules that characterize the growth of bacillus tuberculosis will be missed; but it is interesting and important to note that, though absent, their place is taken by a uniformly mealy condition of the growing mass that impresses one as but a low degree of the conspicuously granular development of bacillus tuberculosis.

For the purposes of this contribution it is needless to dilate upon the development of this group upon special media or their various biological functions under particular conditions of cultivation.* It suffices to say that on solid media favorable to their growth and to that of bacillus tuberculosis there is no likelihood of their being confounded with the latter organism by anyone at all familiar with it.

PATHOGENESIS.

The effect of this group of bacteria on animals is variable, depending upon the animal used, the mode of inoculation, and the quantity of culture employed. When injected beneath the skin of rabbits or guinea-pigs, even in such large doses as from 0.5 to 1 c.c. of an opaque suspension, there is, as a rule, either no result or there is subacute suppuration of the neighboring lymph glands.

If pure cultures be injected into the peritoneal cavity, and the dose be not excessive—*i. e.*, not more than 1 c.c. of an opaque suspension—there is often no result; but if there be injected into the peritoneal cavity of either rabbits or guinea-pigs a small quantity of some such aseptic irritant as sterilized butter, and this be followed by an injection of the bacteria in pure culture, an extensive, sometimes fatal, fibrinopurulent peritonitis usually results.† From this the organism used may be recovered in pure culture. It is important to note that by this procedure, as well as

* For a comparative study of the biology of some of the members of this group, see Pettersson, Berl. klin. Wochenschr., 1899, Jahrg., xxxvi. s. 562.

† It has been suggested that the part played by the butter is not so much that of an irritant, preparing the way for the bacteria by causing points of lessened resistance, as that of a protective, shielding the bacteria from the germicidal action of the living tissues by enclosing them in a fatty envelope. It seems to us that it may be both.

after the injection of butter in which these organisms are accidentally present, the lesions of the peritoneum and the peritoneal surface of the abdominal viscera are often marked by small, round, or oval nodules that have somewhat the appearance of tubercles. Histologically, however, the nodules resulting from this mode of inoculation partake more often of the suppurative than the true tubercular character, though by certain observers they are said to occasionally resemble true tubercles to such an extent as scarcely to be distinguished from them.

By injection directly into the testicles of guinea-pigs, with the view of forcing the bacilli into the abdominal lymphatic circulation, the results may be negative or may result in suppuration at the site of injection. If, on the other hand, sterile butter be injected into the peritoneum of an animal so treated there usually occurs a wide-spread fibrinopurulent peritonitis, marked by nodular formations containing acid-resisting bacteria.

In our experience, the most convenient and satisfactory method of studying the pathogenic possibilities of the members of this group is by intravascular injection. If one inject into the circulation of the rabbit, by way of the vein in the ear, for instance, a moderately large dose (1 to 1.5 c.c.) of a suspension of either of the typical members of the group—viz., “butter bacillus” (Rabinowitsch), “grass bacillus II.” (Moeller), or “timothy hay bacillus” (Moeller)—there appear in from twelve to eighteen days, with a fair degree of regularity, larger and smaller nodules in the internal organs. Some of these—the smaller and younger ones—are often difficult, if not impossible, to distinguish from genuine tubercles of the same age by either macroscopic or microscopic examination; while others—more particularly those that are further advanced—are characterized more by exudative than by proliferative and necrotic tissue changes.* The peculiar distribution of these nodules not only attracts attention, but at once serves to distinguish the process from that following upon the similar inoculation of animals of the same species with genuine tubercle bacilli. In the latter event there is usually an involve-

* For an excellent description of these nodules from the histological stand-point, see Hölscher, *Münchener med. Wochenschr.*, 1901, s. 1483.

ment of all the internal viscera, with the lesions sometimes more numerous and conspicuous in one than another organ, but under all circumstances the lungs are more or less conspicuously affected; while the results following upon the injection of the confusing group of bacteria are in the vast majority of cases confined, singular to say, to the kidneys. Involvement of the lungs, in our experience, has been the exception; for instance, of forty-five rabbits into the venous circulation of which were injected opaque suspensions of these organisms in amounts ranging from 0.5 to 1.8 c.c. only seven developed pulmonary lesions. The animals were killed in—roughly stated—from fifteen to twenty-two days after the operation. Twenty-three of these rabbits were inoculated with Moeller's grass bacillus II., and the lungs of two were involved; eleven received injections of Rabinowitsch's butter bacillus, and suspicious nodules were found in the lungs of one; and eleven were inoculated with Moeller's timothy hay bacillus, and four suffered from lesions of the lungs; while of four rabbits treated in the same way with suspensions of genuine bacillus tuberculosis marked pulmonary involvement was detected in all. In one of these latter animals, killed as soon as ten days after the inoculation, both lungs were found studded throughout with young, easily recognizable miliary tubercles.

We did not in a single instance detect disease of the spleen, liver, myocardium, or diaphragm in animals inoculated with the confusing group of bacteria. In two cases tiny foci of suppuration were found in the brain. These were not subjected to systematic study in serial sections, but in sections taken at random neither acid-resisting bacteria nor bacteria of any sort were detected by microscopic examination. No cultures were made.

On microscopic study of the nodules resulting from injection of *bacillus tuberculosis* one finds, as is well known, miliary and conglomerate tubercles having all the histological peculiarities of those seen in tuberculosis of man, including true giant cell formation, though this is apparently somewhat less common in experimental tuberculosis of rabbits than in tuberculosis occurring spontaneously in human beings. On histological study of the nodules occurring in rabbits after intravenous injection of the acid-resisting bacteria the conditions found are inconstant. Occasionally nodules

are encountered that, both to the naked eye and in their histological structure, are almost indistinguishable from true tubercles, being characterized by central necrosis, giant-cell formation, proliferation of epithelioid and lymphoid cells, and the customary reaction of the surrounding tissues. This is particularly the case in the early stages of development. More often, however, this typical appearance is not encountered. To the naked eye the nodules may to some extent suggest tubercles, but on microscopic study they are found to be characterized more by suppurative and exudative changes than by the proliferative and progressive necrotic reactions peculiar to true tubercular infection, and this difference becomes more and more evident as time advances.

We have observed no tendency to progressive destruction of tissue through the fusion of neighboring nodules, as is so characteristic of tuberculosis; and we have no grounds for believing that the infection becomes general through the transportation of bacilli, by way of the blood or lymph channels, from the foci of disease to other organs and tissues—a very significant difference, as is also noted by Hölscher (*loc. cit.*)—between tuberculosis and this type of infection. When occurring in the lungs—which, as stated, is comparatively infrequent—the lesions observed by us in different animals have varied considerably from one another in their histological peculiarities.

Sometimes the nodules, save for their numerical infrequency and their lack of tendency to dissemination or fusion, could scarcely be distinguished by their individual structure from true tubercles. They were marked at the centre by a kind of necrosis (not typical caseation); there was proliferation of the fixed tissue cells, and marked exudative phenomena or leucocytic invasion were absent. In other cases they were suggestive of tubercles without being very like them. In one case they were characterized so much by proliferation of lymphatic tissue, with little invasion of leucocytes and but limited necrosis, as to leave little room to doubt that it was but a reaction of the normal lymphatic structures of the organ. In another case (intravenous inoculation with timothy bacillus) the lung was marked by several yellow, sharply isolated areas, ranging in size from 2 to 4 mm. in greatest diameter, that were irregular in outline and sharply differentiated from the surrounding healthy

lung tissue. Macroscopically they presented little to suggest tuberculosis; in fact, we were disposed at the autopsy to regard them as either foci of suppuration or as possibly of animal parasitic origin. On microscopic study these areas proved to be very sharply separated from the surrounding healthy lung tissue, and to constitute lesions partaking in some respects of the character of tuberculosis, in others of ordinary granulation tissue. Scattered throughout them were numerous giant cells of various sizes, some lymphoid tissue, and many islands of closely packed polymorphonuclear leucocytes. The most conspicuous feature, however, was the presence of large, stellate mycelia of most unusual appearance. One can best comprehend their structure by likening them to chrysanthemums of microscopic dimensions. In some cases the mycelia were surrounded by epithelioid cells in a more or less active state of proliferation (Fig. 2), giving the picture of giant cells in their formative stage, marked at their centre by the growing fungus; in others, in addition to the proliferating epithelioid cells, the fungus was encompassed by a more or less dense zone of polymorphonuclear leucocytes.

The lesions of the kidneys, which in rabbits are practically constant, may be described as follows: In animals killed twelve to eighteen days after intravenous inoculation of moderately large doses of either of the type organisms the kidneys are found, as a rule, marked by small yellow or gray points. Sometimes these are scarcely larger than a pin-point, are circumscribed, and do not project above the surface of the organ; again, they may be as large as a small pin-head, may be distinctly yellow in color, may stand up prominently from the cortex, and in some cases be so intimately adherent to the capsule that the removal of the latter tears them bodily from the organ. This is not, however, always the case. At times they do not extend to any considerable distance into the organ, while in other cases they may penetrate a millimetre or so into the cortex. On section of the organ they are often found scattered through it, some being confined to the pyramidal portion. To the naked eye only the smallest of these nodules would be mistaken for tubercles. The larger, and particularly those elevated above the surface of the organ, are much more suggestive of miliary abscesses. On microscopic examination, also, some of these nod-

ules in the early stages of their development are almost indistinguishable histologically from true tubercles, but later on they become marked by a degree of leucocytic invasion that converts them into foci having many of the peculiarities of low-grade supuration. In brief, the characteristics of the lesions are those of the so-called "foreign body" tubercles.

In all cases the lesions, from the anatomical stand-point, are like tubercles in that they are isolated nodular points surrounded by more or less unaffected tissue; in that they are characterized in their earliest stages by proliferation of the fixed cells of the tissues, and later by a certain degree and kind of necrosis; and in that they are occasionally marked by giant-cell formation. They are unlike tubercles in the absence of any tendency to fusion, metastases, or dissemination; in the ultimate occurrence of suppuration or organization rather than in progressive destruction of tissue by caseation; and in their occurrence, after introduction into the veins, more often in the kidneys than in any other organ of the body.

From the stand-point of the exciting factors it may be said that when in the tissues of a susceptible animal the tendency of bacillus tuberculosis is to disseminate; the disease caused by it is progressive; metastases are regularly seen, and wherever they occur are characterized by destruction of tissue by caseation. This is not the case with the pseudotubercle bacilli under consideration. They remain where deposited, and though they may develop actively at these points, there is no tendency for their effects to extend beyond very narrow confines or for the bacilli to be distributed in an active state through the blood and lymph streams.

THE EFFECTS OF THE ACID-RESISTING BACTERIA UPON LARGER DOMESTIC ANIMALS.

Through the important investigations of Theobald Smith,²⁹ of Koch,³⁰ and of Ravenel³¹ we have learned that the genuine bacillus of tuberculosis varies in its pathogenic properties according to the source from which it is obtained and according to the animals on which it is tested, cultures obtained from bovines, for example,

being, as a rule of higher degree of virulence than those from man, so that we are beginning, because of this difference, to speak of bovine tubercle bacilli and of human tubercle bacilli.

Through a series of studies during the past decade we have become acquainted with another type of tubercle bacillus—one that affects especially birds. This organism—the so-called bacillus of avian tuberculosis—causes in birds a disease having many of the characteristics of genuine tuberculosis, but it is practically without disease-producing potencies for mammals.

More recently we have learned of the occurrence of tubercle bacilli, or of bacilli like them, in fish and other cold-blooded animals; and from several sources evidence has been presented in favor of the opinion that tubercle bacilli of mammalian origin may become modified in certain respects by a sojourn in the tissues of animals of other classes.

In consideration of these facts we have endeavored to discover if the acid-resisting organisms that simulate bacillus tuberculosis differ likewise in their pathogenic potencies in different species of animals. In this connection it is manifestly of importance to determine their relation not only to the small animals used in the laboratory, but also to those domestic animals that are susceptible to tuberculosis and on which we rely for certain articles of food. With this in view our investigations were made upon hogs and calves. In taking up this phase of our subject the only account of similar studies that came to our notice is that of Sato and Brauer.³² These investigators injected into the peritoneal cavity of each of two calves 100 c.c. of a bouillon culture of an acid-resisting bacillus obtained by them from butter. They suspected its identity with the butter bacillus of Rabinowitsch, though they did not determine this certainly.

One of these calves received only the culture, the other the culture followed by 100 c.c. of sterile melted butter. In the first calf the results were negative; in the second three large tumors partaking of the nature of granulomata were found in the peritoneum at autopsy.

In both cases the animals were killed thirty-five days after inoculation.

Our own experiments and results were as follows :

EXPERIMENTS ON HOGS.

In this series of studies the grass bacillus II. of Moeller was selected not only because it may be taken as a type of the group of acid-resisting bacteria, but because of its habitat and because of the regularity with which pathological lesions suggesting tubercles are produced by it in rabbits and guinea-pigs by particular methods of inoculation.

Four small hogs, about one-third grown, were selected from an unsuspicious source. They were tested with tuberculin, and none reacted.

On November 13, 1901, two cubic centimetres of a thick suspension of grass bacillus II. were injected into the loose cellular tissues of the right groin of each of two of them, and on November 14th the same quantity of the same suspension was injected into the groin of a third, while the fourth pig was kept as a control. The suspension of bacilli injected was made from the entire surface growth from a slanted glycerin-agar culture that had grown for seven days at 37° C. The origin of this culture was a suppurating nodule in the testicle of a guinea-pig produced by injection of grass bacillus II. on October 31st. By culture methods the material injected into the hogs was found to be a pure suspension of the organism under consideration. After injection the four pigs were kept in cages in the laboratory stalls, two in each cage, and were fed daily on measured quantities of suitable food. The food was not sterilized. The animals thrived and at no time evidenced any results of the inoculation.

On January 8th, fifty-five days after inoculation, one of the pigs was killed. At the time of inoculation it had weighed 15.11 kilo.; when killed it weighed 25.49 kilo., so that there had been a gain in weight of 10.38 kilo.

At autopsy there was found a conspicuous deposit of subcutaneous fat. There was no involvement of the subcutaneous lymphatic glands, even of those nearest the site of inoculation, and subsequent microscopic study showed them to be normal. On opening the abdomen the omentum was found marked by a considerable deposit of fat. The small intestines and mesenteric lymphatics were normal. On the rectum, about 5 or 6 cm. from the anus,

were two nodules, one about 5 mm., the other about 15 mm. in diameter. These proved to be abscesses containing creamy pus. They were located beneath the serosa, and in the case of the larger, communicated with the lumen of the intestine. On microscopic examination the pus was found to consist, in the main, of granular débris, a few well-formed polymorphonuclear leucocytes, and ordinary bacilli. No acid-proof bacteria were to be found. By culture methods the bacilli seen proved to be of the colon group. They were not studied in detail. Two guinea-pigs were inoculated with the pus. The one that received the subcutaneous inoculation died in twelve days of general infection. Cultures of bacilli of the colon group were obtained from the spleen and peritoneal cavity. The other, that received an intraperitoneal injection of 1 c.c. of a bouillon suspension of the pus, showed no effects. After twelve days it had gained 60 grammes in weight; it was then killed. The results of the injection as seen at autopsy were negative. The liver, spleen, lungs, heart, and left kidney of the hog presented no deviations from the normal. In the right kidney was a congenital cyst of about the size of a pigeon's egg. It was filled with clear urine. In short, the animal was not in any way affected by the inoculation.

January 23d, seventy days after inoculation, killed small sow. At the time of inoculation she weighed 15.06 kilo.; when killed her weight was 29.28 kilo., making a gain of 14.22 kilo.

Autopsy. Thick deposit of subcutaneous fat. No involvement of subcutaneous lymphatics. Moderate amount of fat in the omentum. Stomach and intestines normal. At one point in the mesentery was a small cluster of lymphatic glands that seemed to be unusual in size, but showed no other change. They were examined microscopically after preservation in Orth's fluid and alcohol, and found to be normal.

Liver, spleen, lungs, and heart normal.

Both kidneys presented a number of small, round, unelevated gray areas of about 1 mm. in diameter. These were plainly visible on the cortical surface after removal of the capsule. They had a little the appearance of young miliary tubercles, but on subsequent microscopic examination they all proved to be collections of lymphoid elements. There was nothing about them to suggest

either proliferative or exudative process, and we therefore regarded them as lymph nodes, probably normal to the organ.

Cultures from the mesenteric lymphatics and from the spleen were negative.

As in the foregoing animal, nothing was found that could properly be referred to the inoculation. In short, the animal appeared normal throughout.

On January 28th the third pig, a small boar that had received a subcutaneous inoculation on November 13th, was found to have gained 10.63 kilo. in the seventy-five intervening days. At no time had it shown any inconvenience attributable to the operation. This, together with the negative results obtained in the first two animals, suggested the advisability of a more radical experiment. On January 28th, therefore, we injected directly into the lung of this animal, and also into that of the control animal, through the thoracic wall, 13 c.c. of a suspension of the same organism. The suspension injected represented the entire surface growth from three well-developed slanted glycerin-agar cultures. The animal experienced no inconvenience either at the time of the injection or afterward. It was killed February 10th, thirteen days after the intrapulmonary injection, and showed at that time a further gain of 0.31 kilo.

Autopsy revealed a thick layer of subcutaneous fat. The subcutaneous lymphatics and the internal organs except the right lung, into which the injection had been made, did not deviate in their appearance from the normal. At the point of puncture of the needle the right lung was adherent to the parietal pleura by several bands of fibrous tissue. Scattered through this tissue, and also at a number of points in the thickened pleura, were isolated yellow points varying in size and shape. The substance of the lung, even in the vicinity of the site of inoculation, showed no nodules, and except immediately beneath the thickened pleura there was no evidence of an inflammatory condition; in brief, it was normal in appearance. On its thoracic surface the diaphragm was adherent to the lung at two isolated points. At three points the new-formed tissue gave the appearance of being acutely inflamed. These were marked by several small more or less circumscribed yellow areas, about which there was a certain degree of

reddening. In all cases these isolated points presented more the appearance of suppurating nodules than of those common to tubercular infection.

On closer study, both by cover-slips at the autopsy and by sections of the hardened tissue, they were found to consist of accumulations of polymorphonuclear leucocytes and enormous numbers of acid-resisting bacteria. In the thickened pleura the acid-resisting bacteria were not only massed in these inflammatory foci, but were widely distributed throughout the communicating lymphatics. In none of the sections were nodules encountered that had the least resemblance to tubercles or to the nodules following upon intravenous injection of rabbits with the acid-resisting organisms.

The bacteria in the tissues presented no characteristic grouping. They were commonly to be seen in dense, irregular clumps, but in no case was actinomyces-like development observed. They were often encountered within the bodies of leucocytes.

On January 23d the fourth pig, which had up to that date served as control, was weighed and found to have gained 13.93 kilo.

On January 28th there was injected directly into its right lung 12 c.c. of a bouillon suspension made from the entire surface growth of three well-developed slanted glycerin-agar cultures of grass bacillus II. There was no immediate result, and the animal was not apparently inconvenienced by the treatment. After fifteen days it was killed, and on weighing before killing it was found to have lost 5.74 kilo. since the inoculation. This is the only one of the four animals on which the general effect of the inoculation was manifested by loss in weight. But it must be remembered that an enormous quantity of material had been injected into its lung.

At autopsy essentially the conditions noted in the third animal were found. There was a marked deposit of subcutaneous and omental fat. The lymphatics neither subcutaneous nor mesenteric were affected. The internal viscera were normal with the exception of the lung into which the injection had been made. This was adherent to the parietal pleura at the point of puncture of the needle, and immediately about this point the pleura was somewhat thickened and marked by a number of elevated yellow areas of from 3 to 4 mm. in diameter. In neither this nor the preceding

animal was there any visible deposit of fibrin or fibro-pus on the pleura.

On microscopic examination of both fresh and hardened preparations the yellow nodules upon the pleura and in the new-formed adhesion bands were found to consist of masses of leucocytes and acid-resisting bacilli. The latter were very inconstant in length, were conspicuously beaded, were often clubbed, and were frequently within the bodies of the leucocytes.

The lung was also adherent at one point to the diaphragm.

By both macroscopic and microscopic examination the lung itself showed no involvement except immediately beneath that part of the pleura in which the inflammatory reaction was most pronounced. Even here there was nothing to be seen in the way of nodules that might be mistaken for tubercles. The change, such as it was, consisted of a limited amount of diffuse inflammatory reaction.

As in the preceding animal, microscopic examination of sections of the thickened pleura and the contained yellow areas showed the latter to consist of sharply circumscribed masses of polymorphonuclear leucocytes and acid-resisting bacilli in large numbers. At many places the latter almost filled the lymphatic spaces in the immediate neighborhood of the suppurating focus. Nowhere was their development or grouping characteristic; they were seen simply as separate rods and threads or as irregular clumps of them.

In both this and the preceding animal Moeller's grass bacillus II. was recovered in pure culture from the nodules on and in the pleura.

Cultures from the site of inoculation of the first two animals that had received subcutaneous injections were negative, and cultures from the internal organs of all four animals (except the right lungs of animals Nos. 3 and 4) were uniformly negative.

From the foregoing we regard it as a justifiable conclusion that the grass bacillus II. of Moeller, which may be fairly regarded as a typical member of the group of pseudo-tubercle bacteria, is practically without pathogenic properties of importance for one of the domestic species that is constantly susceptible to tuberculosis by similar methods of inoculation. It seems unlikely, therefore, that this organism, when gaining access to the bodies of these animals

by the customary portals of accidental infection and in the ordinary numbers, can be regarded as of etiological significance in exciting conditions that may be mistaken for tuberculosis. That such potencies may be exhibited under peculiar predisposing conditions is, of course, not beyond the realm of possibility; but this would probably occur only under an unusual group of conditions—conditions with which we are at this time unacquainted.

INTRAVENOUS INOCULATION OF CALVES.

Six calves were used for this test.

On March 22, 1902, three calves were inoculated intravenously, by way of the left jugular vein, with the *timothy hay bacillus* of Moeller, and three in the same manner with the *butter bacillus* of Rabinowitsch. The amount of culture injected into the circulation of each animal of this series was the entire surface growth from two well-developed slanted glycerin-agar cultures of the organisms used. The cultures were ten days old, having grown for six days at 37.5° C. and for four days at ordinary room temperature. For each injection the bacteria were suspended in from 12.5 to 15 c.c. of sterile bouillon.

Each animal was subjected to the tuberculin test before it was inoculated, and again before it was killed, with negative results in every instance.

None of the animals of this series gave evidence during life of inconvenience from the inoculation. With one exception they all gained in weight at an apparently normal rate. The gains ranged between ten pounds for Calf No. 7 and forty-seven pounds for Calf No. 12. Calf No. 13, the single exception, lost fifteen pounds in the interval of thirty-three days between the date of its inoculation and the termination of the experiment.

Two animals of the series were killed on the twenty-second and four on the thirty-third day after inoculation. In not a single instance did the post-mortem examination reveal the presence of lesions that could be referred to the bacteria with which the animals had been inoculated, and the blood and internal organs were free from living bacteria, as determined by cultures.

The protocols in more or less detail for these animals are as follows :

Calf No. 1.—Inoculated March 22, 1902. 15 c.c. of a bouillon suspension made from the entire surface growth of two glycerin-agar cultures of butter bacillus (Rabinowitsch) were injected into the left jugular vein of this animal. When inoculated the calf weighed 282 pounds; when killed, twenty-three days later, it weighed 296 pounds, making a gain of fourteen pounds. Tuberculin tests before inoculation, and again just before the animal was killed, gave negative results. Twenty-three days after inoculation the animal was killed, and careful examination failed to reveal the existence of any conditions attributable to the treatment the animal had received. By macroscopic examination it was apparently normal throughout. Cultures were negative.

Calf No. 2.—On the same date there was injected into the circulation of this animal the same dose of the same organism as was employed in Calf No. 1. Before inoculation this animal weighed 315 pounds; just before it was killed it weighed 325 pounds, making a gain of 10 pounds in thirty-three days. This calf was subjected to the tuberculin test both before inoculation and immediately before it was killed, with negative results in both instances. It was killed thirty-three days after inoculation.

At autopsy no lesions were found in any of the organs that could be referred to the inoculation; in fact, the viscera were normal throughout, except the right lung, in the upper lobe of which were found a number of small, sharply circumscribed, firm, red and reddish areas, having somewhat the appearance of hepatization. On examining these areas more closely a number of the bronchi were found to be plugged with muco-pus and to contain embedded in this pus actively motile, white, thread-like worms measuring 2.5 to 3 cm. in length, and being of about the thickness of ordinary sewing-thread, sometimes a little thicker. At times only a single one of these worms would be found in a bronchus, while, again, they were present in large tangled masses. It is evident from the foregoing that we have to deal here with the condition of ordinary verminous pneumonia occasionally seen in cattle. Microscopic examination of sections from the diseased portions of this lung failed to reveal the presence of acid-resisting bacteria or of actinomyces, and cultures were negative in so far as the growth of acid-resisting organisms is concerned.

Calf No. 3.—This animal was treated in precisely the same way as Calves 1 and 2, the same organism having been used for the inoculation. At the time of the injection it weighed 363 pounds; just before it was killed, thirty-three days later, it weighed 388 pounds, making a gain of 25 pounds. It was subjected to the tuberculin test both before inoculation and again just before it was killed, with negative results in both instances.

It was killed April 23d—that is, thirty-three days after inoculation—and was found at autopsy to be normal. There were neither lesions attributable to the inoculation nor lesions of any other sort detectable by careful macroscopic examination. Cultures from this animal were negative.

Calf No. 4.—On March 22d, 12.5 c.c. of a bouillon suspension containing the entire surface-growth from two well-developed glycerin-agar cultures of Moeller's timothy hay bacillus were injected into the circulation of this

animal by way of the left jugular vein. At the time of inoculation this calf weighed 229 pounds; just before it was killed it weighed 269 pounds, making a gain of 40 pounds in twenty four days.

At autopsy nothing was discovered in any of the organs that could have been referable to the bacteria injected. Cultures from blood were negative.

Calf No. 5.—This animal was inoculated in precisely the same manner as was Calf 4, the same organism having been used. At the time of the inoculation it weighed 293 pounds; thirty-three days later, when it was killed, it weighed 340 pounds, making a gain of 47 pounds. It was subjected to the tuberculin test both before inoculation and before it was killed, with negative results in both instances. It was killed April 23d, thirty-three days after inoculation.

At autopsy no lesions were found that could be reasonably referred to the inoculation. In both kidneys were a number of irregularly outlined, gray or yellow, depressed areas, having much the appearance of scars. They ranged in size between those not larger than a pin-head to those of from 5 to 6 mm. in their greatest diameter. Sometimes they were superficial, but more often they extended a slight distance into the cortex of the organ. They were much paler than the surrounding tissue; were sharply outlined; there was no apparent reaction of the tissues about them, and in general they were homogeneous in structure, as seen in fresh sections through them. In no particular did they suggest tubercular or suppurative processes. Microscopic examination of these areas revealed a collection of lymphoid cells and some increase of interstitial connective tissue, but no foci of suppuration or caseation. Cultures were negative.

Calf No. 6.—This animal was treated in identically the same manner as Calves 4 and 5 and with the same organism. At the time of inoculation it weighed 300 pounds. It was killed thirty-three days later and found to weigh, just before killing, 285 pounds, making a loss of 15 pounds. It was subjected to the tuberculin test both before inoculation and a few days before it was killed, with negative results in both instances.

It was killed April 23d, thirty-three days after inoculation, and nothing whatever of a pathological nature was found in its internal viscera. Cultures from the blood were negative.

INTRAPULMONARY INOCULATION OF CALVES.

In this test nine animals were used. Five of them were inoculated with Moeller's grass bacillus II., two with butter bacillus (Rabinowitsch), and two with Moeller's timothy hay bacillus.

In all cases the material was injected through the thoracic wall directly into the lung by means of a hypodermic syringe provided with a long needle.

The doses employed varied, the smallest being approximately one-third of a large wire loopful of the surface growth of a

glycerin-agar culture of Moeller's grass bacillus II. suspended in 2.5 c.c. of bouillon, the largest being the *entire* surface growth of two slanted glycerin-agar cultures (ten days old) of butter bacillus and of timothy hay bacillus suspended in from 12.5 to 15 c.c. of sterile bouillon. In no instance were the animals inconvenienced by the injections.

With two exceptions (Calves No. 9 and No. 12) all gained in weight, the gains ranging between one pound in two months for Calf No. 11 and fifty pounds in three months for Calf No. 7. Calf No. 9 lost twenty pounds in three months, and Calf No. 12 two pounds in eighteen days.

As in the case of the preceding series, all the animals of this group were tested with tuberculin before they were inoculated, and all again before they were killed, with negative results in every instance.

The results of the inoculations were negative throughout in so far as the production of conditions simulating tuberculosis is concerned. The protocols of the autopsies in more or less detail are as follows:

Calf No. 7.—November 13, 1901. Injected into the right lung of this calf, weighing 425 pounds, 2.5 c.c. of a bouillon suspension of Moeller's grass bacillus II. The suspension was made by mixing six very large wire loopfuls of well-grown agar-agar cultures of the organism in 20 c.c. of bouillon. The cultures used had uniformly caused pseudo-tubercles when injected into the circulation of rabbits. The calf exhibited no inconvenience from the injection.

It was killed February 14th—*i. e.*, three months after inoculation. At that date it weighed 475 pounds, making a gain of 50 pounds during the three months following the inoculation.

Autopsy. The abdominal viscera were normal. A number of pale points were seen upon the cortices of the kidneys that in general suggested miliary tubercles. A number of these were dissected out, mashed between cover-slips and stained. Microscopic examination showed them to be uniformly free from bacteria. Cultures were also made from a number of them, but no acid-resisting bacilli developed. Sections of the organs preserved in Orth's fluid and in alcohol showed these nodules to be collections of lymphoid cells. No exudative and no necrotic processes were observed in any of them, nor were bacteria detected in any of the sections. We concluded from this that, as in the case of the hogs, these pale areas probably represented normal lymph nodes. This opinion was strengthened by finding similar pale points in fresh calf's kidneys purchased from the butchers in the open market. No

deviations from the normal were detected in any of the other abdominal organs, and none in the heart, pericardium, or left lung.

The right lung, beyond a slight increase of fibrous tissue on the pleura the point of needle puncture, showed no change whatever. At no place were nodules, either suppurative or suggestive of tubercular infection to be detected.

Microscopic examination of the lung at about the point of inoculation showed it to be free from disease; neither necrotic nor suppurative foci being detected anywhere. There was no bronchitis and no increase of the lymphatic tissue about the bronchi.

Calf No. 8.—On the same date there were injected into the right lung of a calf of 257 pounds 2.5 c.c. of the same suspension that was used on Calf 7. This calf was also killed on February 14, 1902. At time of inoculation it weighed 257 pounds, when killed it weighed 275 pounds, making a gain of 18 pounds in the three months interval.

Autopsy. All the internal organs were normal except the inoculated lung. Numerous pale points similar to those found in the kidneys of Calf 7 were found in the kidneys of this animal. These, as in the case of Calf 7, were sterile, and on section were found to be more or less isolated collections of lymph cells, from which all evidence of suppurative or necrotic processes were absent. The pleura of the right lung at the site of inoculation was thickened and marked by several small nodular elevations about 2 to 4 mm. in diameter, that to the touch gave the sensation of hard bodies located some distance beneath the surface.

On microscopic examination of sections through the thickened pleura and the adjacent lung the nodules were found to be in the lung tissue just beneath the pleura and to be due to a localized condition of purulent bronchitis, with considerable increase of peribronchial lymphatic tissue. Some of the bronchi and bronchioles were filled and distended with closely packed polymorphonuclear leucocytes.

No bacteria were found in any of the sections. The surrounding lung tissue was healthy, and there was nowhere to be found anything that either closely or remotely suggested tubercular infection.

Calf No. 9.—On the same date a third calf received an intrapulmonary injection of 5 c.c. of the same suspension of grass bacillus II. When treated this calf weighed 260 pounds, when killed, three months later (February, 14th), it weighed 240 pounds, making a loss of 20 pounds in the interval between the beginning and the termination of the experiment.

Autopsy.—On opening the abdominal cavity the only suspicious condition noted was an apparent enlargement of the mesenteric lymphatic glands. Subsequent microscopic study of these, however, showed them to be free from disease. The abdominal viscera were normal throughout.

The kidneys presented similar pale areas (from 1 to 2 mm. diameter) to those noted in Calves 7 and 8, and, as in the cases of those animals, they proved on microscopic examination to be isolated collections of lymphoid tissue and by cover-slip and cultural methods to be free from bacteria. The heart and left lung were normal.

The right lung, into which the injection had been made, was adherent to the thorax at the site of inoculation by several small bands of fibrous tissue. At its posterior edge it was also adherent over a small area to the diaphragm. To the naked eye it presented, even at the adherent points, little or no deviation from the normal beyond a slight thickening of the pleura. At the point of adhesion to the thorax, entangled in the new-formed tissue, was a nodule about the size of a bean. This proved on closer examination to be a lymphatic gland. There was a moderate invasion of polymorphonuclear leucocytes, some increase of lymphatic tissue, but no isolated areas of suppuration or of caseation, and but little evidence of inflammatory changes in general in this gland.

Sections of the inoculated lung, made as nearly as possible through the point of needle puncture, showed the lung tissue to be unaffected. There were no nodules, no isolated areas of suppuration, and no evidence of diffuse inflammatory changes. The pleura was a little thickened, but was not marked by any isolated points of suppuration or caseation.

Neither in sections of the lung nor pleura were bacteria of any sort found.

Sections through the other portion of the lung that was adherent to the diaphragm gave essentially the same result.

Cultures from the lungs and pale points in kidneys of *all three calves—i. e.*, Nos. 7, 8, and 9—were negative in so far as the development of acid-resisting bacilli were concerned.*

Calf No. 10.—On January 6, 1902, we injected into the left lung of Calf No. 10, 5 c.c. of a very thick suspension of Moeller's grass bacillus II. The suspension was made by mixing in 15 c.c. of bouillon the entire surface growth from three slanted glycerin-agar cultures that had been growing for seventy-two hours at 38° C. The animal was not apparently disturbed by the injection. It was killed March 8th—*i. e.*, sixty days after inoculation. When inoculated it weighed 185 pounds, when killed it weighed 201 pounds, making a gain of 16 pounds since the date of inoculation.

At *autopsy* there was a slight adhesion between the pleura and the chest wall at point of inoculation. This consisted of a slender thread or band of fibrous tissue. The pleura was not apparently thickened and the lung itself was not diseased. The surrounding tissues gave no macroscopic evidence of reaction.

There was nothing beyond that mentioned above worthy of note in the animal. In brief, its internal organs were normal.

Calf No. 11.—On the same date (January 6th) Calf 11 was inoculated in a similar manner with the same dose of the same suspension used on Calf 10.

* By reason of circumstances not under our control these autopsies were made in a high wind and practically in the open air, so that there developed in some of our cultures the ordinary saprophytic bacteria commonly found in the air. We did not, however, as stated above, find any bacteria in the tissues by microscopic examination, and feel justified, therefore, in regarding the growths in our culture tubes as accidental contaminations.

When inoculated it weighed 160 pounds, when killed it weighed 161 pounds, making a gain of 1 pound in sixty days.

Autopsy. On opening the great cavities nothing whatever of an abnormal nature was discovered. There was no adhesion of the inoculated lung to the thorax wall, and it was impossible to find the point at which the lung had been punctured by the needle.

The organs of this animal were so manifestly normal that it was not deemed necessary to preserve them for microscopic study.

Acid-resisting bacteria were not obtained in cultures made at the autopsies on either of the five preceding animals.

Calf No. 12.—Inoculated, March 22, 1902, with butter bacillus (Rabinowitch). Fifteen c.c. of a freshly prepared suspension of butter bacillus (Rabinowitch) were injected through the thoracic wall directly into the right lung of this animal. The suspension injected contained the entire surface growth from two well-developed glycerin-agar cultures of the organism.

At the time of inoculation the animal weighed 485 pounds, when killed, eighteen days later, it weighed 483 pounds, a loss of 2 pounds. The tuberculin test was made both before the animal was inoculated and immediately before it was killed, with negative results in both instances.

The animal was killed on April 9th. On opening the thorax there was found on the surface of the lung at the site of inoculation an irregular, tough, white patch, having much the appearance and consistency of fibrous tissue. It was irregularly oval, about 20 mm. in its greatest diameter, and about 5 mm. thick at its centre. From the centre it became thinner toward the periphery, and finally faded off into an irregular, ragged outer edge. On its under surface it was sharply, though irregularly, differentiated from the underlying lung by a reddish line of demarcation. The lung immediately beneath it appeared normal to the naked eye. At a little distance from this very superficial patch, and so located beneath the pleura as to be invisible from the surface, was another dense mass of whitish tissue of much the same general appearance as that found on the surface. On section this latter was found to be soft and cheesy at the centre, a characteristic not found in the more superficial deposit.

On microscopic examination of cover-slips made with scrapings from these deposits nothing of particular significance was discovered. They revealed only the presence of pus- and tissue-cells, blood-corpuscles and a great deal of granular detritus. No bacteria of any sort were certainly discovered.

Cultures from both points of disease revealed the presence of living butter bacilli (Rabinowitch) in pure culture. There were no lesions of any of the other organs of this animal.

Microscopic examination of the diseased points showed the one more superficially located to be a mass of fibrin and cells with a certain amount of organization in progress at the part next to the lung. It contained no circumscribed foci of either caseation or suppuration, and neither bacilli nor actinomyces were seen, though, as stated, cultures from it revealed the presence of the organism with which the animal had been inoculated.

On similar study of the more deeply located nodule, which we take to be the point at which the bulk of the injected mass was deposited, there were found the characteristics of ordinary granulation tissue. It was marked by a number of larger and smaller foci of necrosis and suppuration, and scattered irregularly through it were a few typical giant cells. These latter were marked at their centre by the presence of smaller and larger clumps of acid-resisting bacteria. In some cases these bacteria stained poorly, in others well. Occasionally a giant cell was seen embedded in a more or less dense mass of polymorphonuclear leucocytes, while, again, such was not the case. Often the giant cells seemed to have been invaded by a few leucocytes which were entangled with the centrally located mass of bacteria. Throughout these sections acid-resisting bacteria in the form of short and long beaded rods and threads (see Fig. 1, c) sometimes irregularly scattered between the cells, sometimes massed together in very large, irregular clumps, were found in enormous numbers. No actinomyces and nothing to suggest them were encountered, neither was there anything about the animal to suggest tuberculosis.

Calf No. 13.—On March 22d, 15 c.c. of a suspension containing the entire surface growth from two well-developed slanted glycerin-agar cultures of butter bacillus (Rabinowitsch) were injected directly into the right lung of this animal.

At the time of inoculation it weighed 227 pounds; on the day it was killed, twenty-six days later, it weighed 263 pounds, being a gain of 36 pounds.

Tuberculin tests before inoculation, and again just before the animal was killed, gave negative results.

At autopsy, April 17th, it was impossible to certainly find the point of entrance of the needle into the lung. The lung was adherent to the chest wall in a number of places by new-formed tissue, and as this was most conspicuous at about the point where we suppose the needle had penetrated, we took it to be one of the results of the injection. There was nothing in the lung itself in this area that could properly be referred to the injection. In the lower lobe of this lung there was found an irregular, sharply circumscribed, fleshy area about 6 c.c. long, by 4 c.c. wide. This area extended well into the lung, was sharply defined, and was surrounded by what was apparently healthy lung tissue. The bronchi scattered throughout this area were filled with yellow muco-pus, and in many of them there were found, sometimes singly, sometimes massed together, tiny, actively motile, thread-like worms of a white color.

On microscopic examination of this lesion we found it to be the ordinary verminous pneumonia. No acid-resisting bacteria were found in it by either microscopic or cultural examination, though a certain number of colonies of bacilli and micrococci that were not resistant to acids appeared in the cultures. There was nothing about the lung that led us to believe the inoculation had had any serious effect. The other organs of the animal were to the naked eye normal. Cultures were negative in so far as acid-resisting bacteria are concerned.

Calf No. 14.—On March 22d, 12.5 c.c. of a suspension containing the entire surface growth from two well-developed slanted glycerin-agar cultures of Moeller's timothy hay bacillus were injected directly into the right lung of this animal.

At the time of injection the animal weighed 275 pounds; on the day it was killed, eighteen days later, it weighed 295 pounds, making a gain of 20 pounds.

Tuberculin tests before inoculation, and before the animal was killed, gave negative results.

At autopsy the only lesion found that could reasonably be referred to the inoculation was a tough, more or less raised area of about 8 mm. in diameter at the point where the needle entered the lung. This on microscopic examination proved to be only a thickening of the pleura with a few isolated areas of softening scattered through it. The remaining organs of this animal showed no pathological lesions. Cultures did not reveal the presence of acid-resisting bacteria.

Calf No. 15.—On March 22 the entire surface growth from two well-developed slanted glycerin-agar cultures of Moeller's timothy hay bacillus suspended in 12.5 c.c. bouillon was injected into the right lung of this animal through the thoracic wall.

At the time of inoculation the animal weighed 354 pounds; on the day it was killed, twenty-six days later, its weight was 384 pounds, making a gain of 30 pounds.

Tuberculin tests were negative both before inoculation and before the calf was killed.

At autopsy a few adhesions were found to have formed between the lung and the chest wall. Over the pleural surface of the lung, in the neighborhood of the point of inoculation, were numerous tiny transparent elevations having somewhat the appearance of deposits of fibrin. They were entirely superficial. Beyond these fibrin deposits we were unable to detect any disease whatever of the lung, notwithstanding the fact that macroscopic sections were made over a comparatively large portion of the lung in the neighborhood of where we presume the bacteria had been deposited.

The heart, liver, and spleen of this animal revealed no pathological lesions. Upon the peritoneal surface of the intestines were numerous deposits of loosely adherent fibrin. On examining the peritoneal cavity closely a number of thread-like actively motile worms of about one and a half inch to two inches in length were found.

Microscopic examination of the deposits of fibrin on the surface of the lung showed them to consist of fibrin and cells. No bacteria were detected in them. There was nothing anywhere in this animal to suggest tuberculosis, or even to indicate that the injection had been the cause of a pathological lesion that in any way inconvenienced the animal. Cultures: no acid-resisting bacteria found.

The outcome of the foregoing experiments demonstrates, we believe, the incapacity of the organisms employed to cause in

calves lesions that might be mistaken for those of a tuberculous nature.

From both intravenous and intrapulmonary inoculation there was either no result at all or there occurred only local processes of an ordinary inflammatory character.

In the light of these results we believe that the presence of acid-resisting bacteria (other than tubercle bacilli) in dairy products may be interpreted as accidental contaminations from the surroundings under which the work is performed, and not as evidence of disease caused by these organisms in the animals from which the products are obtained.

THE BOTANICAL RELATION BETWEEN THE ACID-RESISTING BACTERIA AND BACILLUS TUBERCULOSIS. NOMENCLATURE, CLASSIFICATION, ETC.

In still other particulars we encountered both points of resemblance and of difference between these acid-resisting organisms and bacillus tuberculosis, notably in their modes of development in the affected tissues. As has been already beautifully demonstrated by Babes and Levaditi,³³ Friedrich,³⁴ Friedrich and Nösske,³⁵ and Schulze,³⁶ the growth of bacillus tuberculosis in the tissues of susceptible animals often assumes the character of typical actinomyces. This result has been obtained by the authors named through several methods of inoculation—by Babes and Levaditi through subdural inoculation of rabbits, by Friedrich and Nösske through intra-arterial injection of the same animal, and by Schulze through direct inoculation of the internal viscera as well as by the methods of the other investigators.

In our experiments we were able to confirm these observations by the simple method of intravenous injection (see Fig. 3). While we have observed the "ray fungus" form of growth of bacillus tuberculosis in a number of instances, it has not been by any means a common characteristic. We often found it necessary to examine many series of sections through tissues studded with tubercles before we encountered a single mycelium suggesting actinomyces. In the majority of cases the tubercles contained only irregularly clumped or diffusely scattered rods having all the well-known characteristics of bacillus tuberculosis.

Early in the course of our work with the acid-resisting group our attention was arrested by clusters of club-shaped bodies within the nodules in the tissues that in arrangement, general appearance, and type of tissue surrounding them bore many points of resemblance to actinomyces as one sees them in the actinomycotic tumor. At first they were encountered only occasionally in sections selected at random, but by systematic study of serial sections it soon became evident that this is the normal mode of development of these organisms in the tissues, and that in each nodule it is possible to detect, by that method of examination, the presence of one or more such mycelia. While the general character of these mycelia is the same for the three organisms studied by us—*i. e.*, all appear as more or less typical actinomyces, having a central mass of closely interwoven, fine, granular threads surrounded by a zone of radially arranged, elongated, club-shaped hyphæ—there are nevertheless interesting individual differences. For instance, the actinomyces developing from the butter bacillus (Rabinowitsch) and from the grass bacillus II. (Moeller), which do not differ materially from one another (see Fig. 4), are often (not always) much smaller, more delicate, and less numerous than are those from the timothy hay bacillus (Moeller), which are, as a rule, much larger and apparently more prone to “involution” changes. We say “involution changes,” for this is the only expression that appears appropriate to some of the extraordinary appearances encountered in the actinomyces of this fungus. Now and again hyphæ of enormous proportions, relatively speaking, will be seen that are apparently split or creased in such a manner as to produce distinct spines or serrations along their edges and over the surfaces, causing at times the appearance of a miniature barley head or a tiny pine cone. (See Fig. 1, *d.*) The neighboring hyphæ may be of the ordinary club shape or may extend into the tissues as long, slender, ribbon-like projections. Sometimes they are homogeneous in structure, sometimes irregularly segmented. In several instances we were able, by appropriate methods of staining, to distinguish in the large club-shaped hyphæ a central thread or axis and an enveloping mass having different micro-chemical reactions (see Fig. 1, *e*), just as was done by Boström³⁷ in his exhaustive study of the ray fungus occurring in actinomycosis.

When in the diseased kidneys, the mycelia developing from the three organisms are much more alike than when in the lungs, though often in the kidneys the large, irregular timothy hay mycelia will be encountered. The lungs appear to offer conditions somewhat more favorable to the typical actinomyces development than do the kidneys. In one of our cases (mentioned above) the diseased areas in the lungs were marked by numerous isolated mycelia of an unusually beautiful appearance, consisting of long, thick, club-shaped, and ribbon-like hyphæ that radiated into the surrounding tissues from a small, central ring of short, oval, deeply staining bodies. (See Fig. 2.) At times these growing masses were surrounded by proliferating epithelioid cells in such a way as to suggest developing giant cells, while at other points they were completely surrounded by dense zones of polymorphonuclear leucocytes; between these extremes all grades of tissue reaction could be detected. The general character of these mycelia was so unusual as to lead us to suspect that they were possibly the growth of some fungus other than that with which the animal had been inoculated, and that they were, therefore, the result of an accidental infection; but their appearance in the typical nodules in the kidneys of this animal as well as in the lung, and the fact that the so-called timothy hay bacillus was obtained in pure culture from the affected areas, did not contribute to strengthen this opinion. However, a number of efforts were made to reproduce the picture; and while we have in three instances succeeded in doing so in part—sufficient to warrant the belief that our first case was not a mixed infection—we have only once duplicated the condition seen in our first case. In the majority of nodules the mycelia, instead of conforming in structure to those depicted in Fig. 2, were in general more like those shown in Fig. 5. Between these extremes all gradations from one to the other type could be encountered.

Occasionally the mycelia of the timothy hay bacillus were indistinguishable in size and configuration from those of grass bacillus II. and butter bacillus. In one case the growth of the butter bacillus was in the form of the larger mycelia often seen in the development of the timothy hay bacillus. Whether there is a direct connection between the type of mycelium and the character

and extent of the pathological lesion cannot with certainty be decided; but in our experience we have been impressed by the fact that the lesions resulting from the use of the timothy hay bacillus have, as a rule, been much more marked, more numerous, and more often encountered in the lungs than was the case with those caused by the other organisms used in these studies.

While a diffuse growth of these bacteria in the form of short rods, single and in irregular clumps, is now and then observed in the diseased tissues, it is far from constant. One may often examine a complete series of sections through a nodule or group of nodules without detecting any evidence of bacterial development except that given by the presence of the actinomyces. Whether these develop from a single cell or group of cells deposited in the tissues as a result of the method of inoculation we cannot say.

In contrast to this condition it is of interest to note that when large quantities of these organisms are injected directly into the tissues at a single point there is no development as actinomyces. They are seen instead as single rods and clumps of rods scattered irregularly throughout the affected area, just as is usually the case with bacillus tuberculosis in actively developing tubercles. In such cases the rods and threads are conspicuous for their granular structure, appearing often as long and short almost colorless tubes or sheaths, in which deeply stained round points are irregularly distributed. (See Fig. 1, c.)

It must be understood that our observations in connection with the actinomyces development of the acid-resisting bacteria are not unique.* In an elaborate investigation of this subject Lubarsch³⁸ called attention to this mode of growth. He found, in so far as we can judge from his description and illustrations, practically the same conditions that have been noted by us, with the exception, perhaps, of the remarkable development depicted in Fig. 2. His method of investigation differed somewhat from ours, consisting in the main of intra-arterial injection and of direct inoculation of the internal viscera, as was done by Schulze (*loc. cit.*) in his work with bacillus tuberculosis. The organisms employed by Lubarsch, in addition to those used by us, were the bacillus of avian tuberculosis, "modified" bacillus tuberculosis, bacillus of fish tuberculosis, Moeller's mist bacillus, Petruschky's strep-

tothrix, streptothrix Eppinger, bacillus mallei, and bacillus diphtheriæ.

Actinomyces development was not observed in the case of bacillus mallei, bacillus diphtheriæ, and the streptothrix of Petruschky. With all the rest it was a common characteristic of their growth in the tissues.

In 1884 Petrone³⁹ expressed the belief that bacillus tuberculosis represents a phase of development of a parasite intermediate, morphologically, between the micromycetes and the schizomycetes. In 1888 Metschnikoff⁴⁰ remarked that what we are accustomed to see and designate "bacillus tuberculosis" is but a state in the life-history of a parasite of higher organization than the bacilli; and in 1893 we find Hueppe,⁴¹ as a result of Fischel's⁴² investigations, giving as his opinion that "the so-called tubercle bacilli are only the parasitic adaptation form of a fungus-like, pleomorphic microbe."

Through the investigations especially of Fischel (*loc. cit.*), Coppen-Jones,⁴³ Bruns,⁴⁴ Babes and Levaditi (*loc. cit.*), Friedrich and Nösske (*loc. cit.*), and Schulze (*loc. cit.*) the gist of these opinions—viz., that "bacillus" tuberculosis is a misnomer—has received sufficient support to warrant acceptance. It has been demonstrated that, in addition to the actinomyces-like development in the tissues of animals, the exciter of tuberculosis under artificial cultivation develops in the form of elongated, interwoven, branched threads—characteristics that we know are not peculiar to bacilli, strictly speaking. From the studies of a number of other investigators, especially Pettersson⁴⁵ and Moeller,⁴⁶ similar long, thread-like branching forms are to be seen in artificial cultures of some of the acid-resisting group.

From the evidence at hand, of which the foregoing citations represent only a small part, there can be little doubt of a botanical relationship between the so-called "bacillus" tuberculosis, the so-called acid-resisting "bacilli," the ray fungi, known to us in connection with actinomycosis of man and animals, and some of the so-called streptothrices concerned in the condition known as pseudotuberculosis. Nor can there be much difference of opinion as to the inaccuracy of the designation "bacillus" for these organisms. But how, then, shall they be generically designated? In

an elaborate thesis upon the ray fungi Lachner-Sandoval⁴⁷ presents a strong plea for their classification as actinomyces. He calls attention to the fact that the term "streptothrix," frequently used in recent times for some of the members of this group, has long been used by botanists to designate a class of fungi that have little or nothing in common with the organisms with which we are concerned, and that these latter organisms—*i. e.*, tubercle bacilli, acid-resisting bacilli, the so-called streptothrices of the medical bacteriologists, and the actinomyces of actinomycosis—cannot, therefore, be properly included with the streptothrices. Because of their many points of resemblance to the well-known actinomyces of actinomycosis in some cases, and because of the suggestive stellate (though less characteristic) growth in the tissues in other cases, he suggests the term "actinomyces" as the designation for the entire group—a suggestion that we are disposed to regard as appropriate to the case in point.

THE PRESENCE AND SIGNIFICANCE OF ACID-RESISTING BACTERIA IN BUTTER.

The detection of acid-resisting bacteria that may be mistaken for bacillus tuberculosis upon fodder and many cereals; in the soil and manure about barns and stables; in the secretions about the genitalia and udders of milk-producing animals, and in the milk and butter from such animals, evidently demands for them a special consideration by those who are responsible for the sanitary control of dairy industries.

Our knowledge of the occurrence of these organisms in dairy products dates from the observations of Petri¹³ and of Rabinowitsch.¹⁴

Though bacillus tuberculosis had been demonstrated in milk and in butter by many competent observers, it was nevertheless somewhat disturbing to learn through the studies of Obermüller⁴⁸ that he had found them in all of fourteen samples of butter examined by him from a single source in Berlin. While commenting upon Obermüller's results, with the view of dissipating such apprehension as might be caused in the minds of readers, Petri¹³ makes a preliminary announcement of results obtained by him in the

Imperial Health Bureau at Berlin. He found that in about 30 per cent. (!) of over a hundred samples of butter examined were tubercle bacilli certainly found, and that in about 60 per cent. of the samples there was present an organism which might be readily confounded with bacillus tuberculosis. In his completed contribution it appears, to be precise, that 102 samples of butter were subjected to careful study by both microscopic and inoculation methods; that of these 32.3 per cent. contained genuine tubercle bacilli capable of causing tuberculosis when inoculated into guinea-pigs, while in 52.9 per cent. of them was a "new" organism ("stäbchen"), which in stained preparations bore an extraordinary resemblance to bacillus tuberculosis, and which when injected with butter into animals caused lesions that were remarkably like those of genuine tuberculosis. In 16.7 per cent. of the samples genuine tubercle bacilli were found alone; in 37.2 per cent. only the "new" bacterium was present, while in 15.7 per cent. the two organisms were found together.

Of sixty-four samples of milk examined 14 per cent. contained genuine tubercle bacilli, and 6.3 per cent. the "new" bacterium.

In the case of both milk and butter the results given were reached through animal inoculation, 408 guinea-pigs having been inoculated with the butter and 478 with the milk.

He expresses the opinion, in the light of these results, that the simple microscopic examination of butter and milk with the view of identifying bacillus tuberculosis by its micro-chemical staining reaction is untrustworthy.

Almost coincidently with Petri's preliminary announcement appeared a contribution from Rabinowitsch¹⁴ which has also attracted a great deal of attention as well as done much to further elucidate the subject. In this paper she states that Koch had long suspected the existence in dairy products of bacteria that might be and that probably had been mistaken for true tubercle bacilli. It was this thought that led him to suggest her investigation. Under precautions against which no objections could be raised Rabinowitsch examined by microscopic, cultural, and inoculation methods thirty samples of butter purchased in the open markets of Berlin and fifty in Philadelphia. In twenty-three samples (ten in Berlin and thirteen in Philadelphia)—i. e., 28.7

per cent. of the total number examined—acid-resisting bacteria were found which, in morphology and staining reactions, more or less closely resembled *Bacillus tuberculosis*. In their cultural peculiarities these organisms were readily distinguishable from *Bacillus tuberculosis*, but when injected with butter into the peritoneum of guinea-pigs they possessed the property of causing nodular lesions that both to the naked eye and by microscopic examination had some of the characteristics of true tubercles. In this investigation she failed to find tubercle bacilli in a single one of the eighty samples of butter examined; but in a subsequent study of butters obtained from nineteen different places in Berlin the samples repeatedly obtained from one source were always found to contain them, while those from the remaining eighteen sources were regularly free from them.

As the place from which the tuberculous samples were regularly obtained was one of the best-known butter shops in Berlin, she suspects that the discrepancy between her results and those of some of the other investigators, who encountered tubercle bacilli much more frequently in butter, may be in part explained by their having obtained their samples from the shop from which she regularly secured the tuberculous butter.

The investigations of Herbert,¹⁷ conducted in Baumgarten's laboratory at about the same time that Petri and Rabinowitsch were engaged in their studies, are of no less interest and importance than those of Petri and of Rabinowitsch. He examined 126 samples of butter from various sources for the presence of tubercle bacilli, and failed to detect them in a single instance. In only five per cent. of one hundred samples from various sources in Würtemberg were acid-resisting "pseudo-tubercle" bacilli present, while in ten out of twenty (50 per cent.) of the Berlin samples and four out of five (80 per cent.) samples from Munich they were found.

While the works just cited by no means deny the occasional occurrence of tubercle bacilli in butter, they nevertheless indicate their comparative infrequency in samples selected at random. On the other hand, the studies of Rabinowitsch leave no doubt, as had already been emphasized by Obermüller, that butter from particular sources may now and again be encountered in every sample

of which the living virus of tuberculosis may be regularly demonstrated. For our present purpose it is also important to note that though tubercle bacilli were seldom encountered, except in Petri's studies, the acid-resisting, confusing species, though far from regular in their occurrence or distribution, were much more common, having been encountered, as said, by Petri in about 53 per cent. of samples, by Rabinowitsch in about 23 per cent., and by Herbert in from 5 per cent. to 80 per cent., according to the locality from which the samples came.

With the view of acquainting ourselves with the acid-resisting bacteria under their presumably saprophytic existence and of securing material with which to pursue our work, we conducted a series of studies upon butters purchased in Philadelphia.

Fifty samples in all were examined. Thirty-five of these were butters from public creameries, fifteen from private dairies.

Of the fifty samples examined seventeen could properly be designated as good butter, sixteen as medium, and seventeen as markedly poor butter.

Our methods of experimentation were as follows: The samples of butter were melted at from 37° C. to 39° C., and the injections into animals were made as follows:

From one sample 1 c.c. of the whole (mixed) melted butter was injected into the peritoneal cavity of each of four guinea-pigs.

From fourteen samples 1 c.c. of the sediment that collects in the melted butter on standing was injected into the peritoneal cavity of each of twenty-eight guinea-pigs, two pigs being used for each sample.

From thirty-five samples a sediment was obtained by centrifugalization after the plan suggested by Obermüller, and of this sediment from 1 to 1.8 c.c. was injected into the peritoneal cavity of guinea-pigs. One hundred and four animals were used in the investigation.

For each of forty-eight of the samples of butter two animals were used, while for each of the remaining two samples four animals each were used.

Of the 104 animals thus treated thirty-one died of general infection in from two to nineteen days after injection. One died in thirty-four days after the injection, and its peritoneum was

marked by nodular growths from which acid-resisting bacteria were isolated. In the loose tissues at the angle between each kidney and its adrenal body, and adherent to the capsule of the kidney, was an abscess of about 3 mm. in diameter. By both microscopic and cultural methods acid-resisting bacteria were demonstrated in these abscesses.

Five of the remaining seventy-two animals that were killed in from forty-three to sixty-eight days after inoculation showed the presence of nodular growths in the peritoneum in which acid-resisting bacteria were detected by microscopic examination and from which they were isolated in pure cultures; while of the balance, sixty-seven in all, sixty-six were killed in from thirty-one to sixty-nine days after the inoculation, and found to be devoid of lesions that could have been mistaken for tubercles or that would have been likely to have caused the death of the animals; and one died thirty-eight days after inoculation, the autopsy revealing dense adhesions of the intestines to one another and to the liver and gall-bladder. No nodules of any sort were detected. By both microscopic and cultural methods of examination the peritoneum was found to be sterile.

The nodules found in the peritoneum of the animals from which acid-resisting bacteria were cultivated may fairly be said to have suggested tubercles to the naked eye only remotely. They were nodular and were isolated, it is true; but they were of a yellowish or whitish appearance, were less sharply circumscribed than tubercles, were confined to the peritoneum, showing little or no tendency to invade the deeper structures of the organs or to metastasis throughout the body, and were generally devoid of the clear, pearly appearance seen in genuine young tubercles. On microscopic examination they were all so characterized by the invasion of leucocytes and by the absence of caseation, giant-cell formation, or the necroses typical of tuberculosis that we could see no reasonable excuse for mistaking them for tubercles. In two of these cases, in addition to the tiny nodules, small, unmistakable abscesses were present; in one of these cases there was a small, circumscribed focus of suppuration in the loose tissues about the right kidney, and in the other there was a similar abscess in the left testicle.

Six of the ten animals that had been inoculated with the five samples of butter in which acid-resisting bacteria were detected presented the nodular lesions noted above. Of the remaining four animals inoculated from these samples negative results were obtained in one, and three died of acute infection.

Without entering into details it will suffice to say that throughout our experiments the deaths due to acute general infection were referable to the activities of *staphylococcus pyogenes aureus*, bacilli of the colon and proteus groups, and to *streptococcus pyogenes*. In the case of one sample of butter the two animals inoculated with it were killed fifty-two days after the intraperitoneal injection, and the only organisms found in their tissues were yeast cells. There were no suspicious nodules detected. In the majority of samples examined by us yeast cells were found by direct microscopic examination of the sediment.

In four of the five samples of butter that caused nodular lesions of the peritoneum acid-resisting bacteria were found by microscopic examination of the sediment. In one they were missed. In another sample acid-resisting bacteria were detected microscopically in the sediment, but of four animals inoculated from this sample three died of acute infection; while the remaining one, killed forty days after inoculation, was devoid of pathological lesions.

In none of the fifty samples of butter examined was the presence of genuine tubercle bacilli demonstrated by intraperitoneal inoculation of guinea-pigs, though it must be remembered that a fairly large proportion of the animals—*i. e.*, 29.8 per cent.—died of acute infections; some of them before tubercle bacilli that might possibly have been present could have exhibited their specific disease-producing properties.

Three of the five samples of butter in which acid-resisting bacteria were detected were from private dairies, while two were from public creameries.

In the selection of our samples for investigation care was taken to secure them from as many different sources as possible. They were purchased in the open market, sometimes from retailers, at others from the wholesale dealers. They were all purchased in different parts of the city, and, so far as we can say, no two samples were made at the same place.

In brief, of fifty samples of butter from various sources examined by us five samples, or 10 per cent., contained acid-resisting bacteria that simulated bacillus tuberculosis in some of their staining reactions; that suggested that organism by certain morphological peculiarities; and that were capable of causing nodular lesions when injected with butter into the peritoneum of the guinea-pig.

Genuine tubercle bacilli were discovered in none of them.

Organisms capable of causing acute peritonitis or general infection were present in twenty-one of them.

From one of the five samples containing acid-resisting bacteria two varieties of those organisms, easily distinguished from one another, were isolated. In one of the samples the acid-resisting organism, while being a rod with resistance to decolorization by weak acids, was only occasionally suggestive of bacillus tuberculosis in morphology. In culture it was unlike any of the group that we have had an opportunity to study, developing on glycerin-agar as a moist, slimy layer of an orange color that remains throughout of a distinctly mucoid consistency. At no time does it present the dry, granular, wrinkled appearance of the type members of this group.

Only four of the cultures isolated by us possess the group-characteristics common to such typical members as the grass bacillus II. and timothy bacillus of Moeller and the butter bacilli of Petri and of Rabinowitsch.

We have not studied the cultures isolated by us in all details, since for the purposes of this paper it seemed sufficient to determine the possession by them of such characteristics as would justify their inclusion in this group. We have as yet made no direct effort to identify them with the several varieties described by other observers.

The results of intravenous inoculation of rabbits with large doses of pure cultures of three of the organisms isolated by us were negative after thirty to thirty-three days.

The microscopic appearance of the acid-resisting bacteria in the samples of butter in which we found them was (when they were stained by the Gabbet method or by the Koch-Ehrlich method, and decolorized by 5 per cent. sulphuric acid in water) sufficiently suggestive of bacillus tuberculosis to render the differ-

entiation from that organism by the microscope a matter of difficulty if not of impossibility.

CONCLUSIONS.

1. That the majority of the acid-resisting bacteria may be distinguished from true tubercle bacilli by their inability to resist decolorization by a 30 per cent. solution of nitric acid in water.

2. That some of the acid-resisting bacteria are capable of causing in rabbits and guinea-pigs nodular lesions suggestive of tubercles; that these lesions, while often very like tubercles in their histological structure, may nevertheless usually be distinguished from them by the following peculiarities:

(a) When occurring as a result of intravenous inoculation they are always seen in the kidneys, only occasionally in the lungs, and practically not at all in the other organs.

(b) They constitute a localized lesion, having no tendency to dissemination, metastasis, or progressive destruction of tissue by caseation.

(c) They tend to terminate in suppuration or organization rather than in progressive caseation, as is the case with true tubercles.

(d) They are more commonly and conspicuously marked by the actinomyces type of development of the organisms than is the case with true tubercles, and these actinomyces are less resistant to decolorization by strong acid solutions than are those occasionally seen in tubercles.

3. That by subcutaneous, intravenous and intrapulmonary inoculation of hogs (4) and calves (15) the typical members of the acid-resisting group are incapable of causing lesions in any way suggestive of those resulting from similar inoculations of the same animals with true tubercle bacilli.

4. That though occasionally present in dairy products, they are to be regarded as of no significance, etiologically speaking, but may be considered as accidental contaminations from the surroundings and not as evidence of disease in the animals.

5. That the designation "bacillus" as applied to this group of bacteria and to the exciter of tuberculosis is a misnomer; they are more correctly classified as actinomyces.

In closing this contribution we wish to express our obligation and thanks to Dr. Alfred Moeller, of Belzig; Dr. Lydia Rabinowitsch-Kempner, of Berlin; Professor Hueppe, of Prague; Dr. Norman McL. Harris, of Baltimore, and Drs. Ravenel and Kneass, of Philadelphia, for their courtesy in sending to us cultures with which to carry out our investigations.

REFERENCES.

1. Zahn. Med. Cor. Bl. d. Würtemb. ärzt. Vereins, 1884, p. 129.
2. Alvarez und Tavel. Arch. de phys. norm. et path., 1885, 3 s., vi. p. 303.
3. Matterstock. Sitzungsber. d. phys. med. Gesellsch. zu Würzburg, 1885, p. 65.
4. Bitter. Virchow's Archiv, 1886, cvi. p. 209.
5. Cramer. Sitzungsber. de phys.-med. Soc. zu Erlangen, December, 1882.
6. de Giacom. Fortschr. d. Med., 1883, i. p. 145.
7. Fraenkel, A. Berl. klin. Wochenschr., 1898, pp. 246, 880.
8. Pappenheim. Ibid., 1898, p. 809.
9. Rabinowitsch. Deutsche med. Wochenschr., 1900, p. 257.
10. Laabs. Inaug. Diss., Freiburg, 1894, Br. i.
11. Moeller. Zeit. f. Hyg. u. Infektionskr., 1889, xxxii. p. 211.
12. Dietrich. Berl. klin. Wochenschr., 1899, p. 189.
13. Petri. Hygienische Rundschau, 1897, p. 811. Arb. a. d. Kais. Gesamte, Band xiv. p. i.
14. Rabinowitsch. Zeit. f. Hyg. u. Infektionskr., 1897, xxvi. p. 90. Deutsche med. Wochenschr., 1897, No. 32. Ibid., 1899, p. 5.
15. Hormann and Morgenroth. Hyg. Rundschau, 1898, p. 217. Ibid., 1898, p. 1081.
16. Grassberger. Münchener med. Wochenschr., 1899, pp. 341, 382.
17. Herbert. Arb. a. d. path. anat. Institut zu Tübingen, 1899, iii. p. 207.
18. Weissenfeld. Berl. klin. Wochenschr., 1899, p. 1053.
19. Korn. Archiv f. Hygiene, 1899, xxxvi. p. 57. Cent. f. Bact., etc., Abt. I., 1899, xxv. p. 532.
20. Ascher. Zeit. f. Hygiene u. Infektionskr., 1899, xxxii. p. 329.
21. Coggi. Gior. d. Royal Soc. Ital. d'Igiene, 1899, No. 7.
22. Herr and Beninde. Zeit. f. Hygiene u. Infektionskr., 1901, xxxviii. p. 152.
23. Tobler. Ibid., 1901, xxxvi. p. 120.
24. Moeller. Deutsche med. Zeitung, 1898, p. 135. Deutsche med. Wochenschr., 1898, p. 376. Therap. Monatssch., 1898, xii. p. 607. Cent. f. Bakt., etc., Abt. I., 1899, xxv. p. 369.
25. Severin. Cent. f. Bakt. etc., Abt. II., 1895, p. 97.
26. Capaldi. Quoted from Rabinowitsch, Zeit. f. Hyg. u. Infektionskr., 1897, xxvi. p. 105.
27. Ferran. Cent. f. Bakt., etc., Abt. I., 1897, xxii. p. 483.
28. Cowie. Journ. Exp. Med., 1900, v. p. 205.
29. Smith, Theobald. Trans. Assoc. Amer. Phys., 1896, xi. p. 75.

30. Koch. Trans. of British Congress on Tuberculosis, London, 1901.
31. Ravenel. Ibid. ; also Univ. of Penna. Med. Bull., 1901, xiv. p. 238.
32. Sato and Brauer. Zeit. f. Fleisch u. Milch-hygiene, 1901, pp. 11-15.
33. Babes and Levaditi. Arch. d. med. Exp. e d'Anat. path., 1897, ix. p. 1041.
34. Friedrich. Deutsche med. Wochenschr., 1897, p. 653.
35. Friedrich und Nösske. Ziegler's Beiträge zur path. Anat. u. zur allg. Path., 1899, xxvi. p. 470.
36. Schulze. Zeit. f. Hyg. u. Infektionskr., 1899, xxxi. p. 153.
37. Boström. Ziegler's Beiträge zur path. Anat. u. zur allg. Path., 1890, ix.
38. Lubarsch. Zeit. f. Hyg. u. Infektionskr., 1899, xxxi. p. 187.
39. Petrone. Cited by A. Coppen-Jones. Cent. f. Bakt. u. Parasitenkunde, 1896, xx. p. 394.
40. Metschnikoff. Virchow's Archiv, 1888, cxiii. p. 63.
41. Hueppe. Verhandl. d. Gesell. Deutscher Naturforscher u. Aerzte, 1893, Th. i. p. 157.
42. Fischel. "Untersuchungen über die Morphologie und Biologie des Tuberkulose Erregers" (Monograph), Wien (Braumüller), 1893; also Berl. klin. Wochenschr., 1893.
43. A. Coppen-Jones. Cent. f. Bact. u. Parasitenkunde, 1895, xvii. p. 1. Ibid., 1896, xx. p. 393.
44. Bruns. Ibid., 1895, xvii. p. 817.
45. Pettersson. Berl. klin. Wochenschr., 1899, xxxvi. p. 562.
46. Moeller. See 24; also Trans. of British Congress on Tuberculosis, London, 1901.
47. Lachner-Sandoval. "Ueber Strahlenpilze. Eine bacteriologisch-botanische Untersuchung," 1898, Strassburg (Ludolph Beust).
48. Obermüller. Hygienische Rundschau, 1897, p. 712.





FIG. 1.

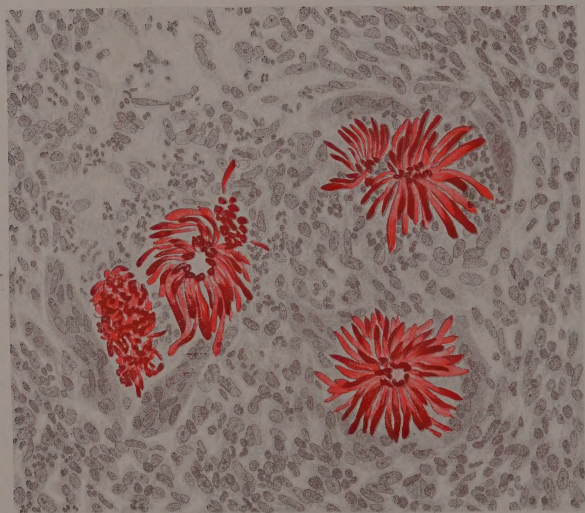


FIG. 2.

K.M.M.

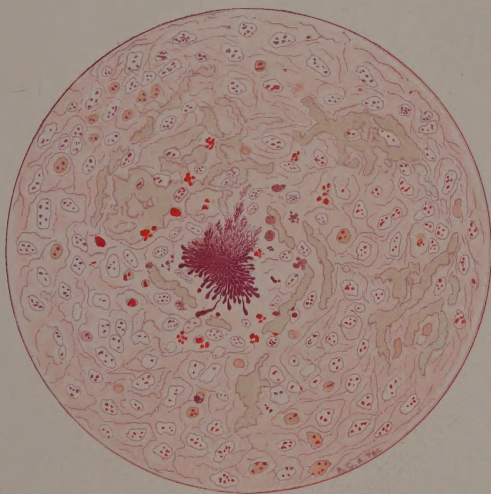


FIG. 3.

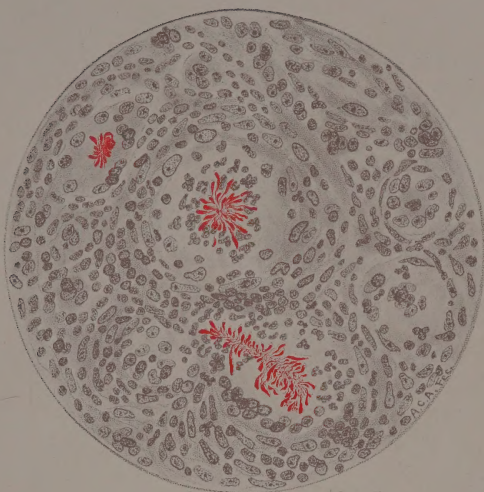


FIG. 4.

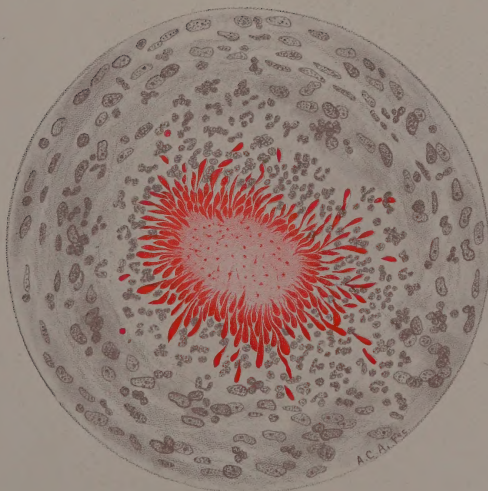


FIG. 5.

EXPLANATION OF ILLUSTRATIONS.

FIG. 1.—Morphological variations.

(a) Pure culture on agar-agar, showing bacillary forms homogeneously stained.

(b) Pure culture on agar-agar, showing bacillary forms in an extremely granular condition.

(c) Long and short markedly granular forms sometimes seen in the tissues.

(d) Involution forms occasionally seen in the actinomyces developing in the tissues after intravenous injection of the timothy hay bacillus.

(e) Showing composite nature of the large club-shaped hyphæ from the timothy hay mycelia. Stained by the method suggested by Mallory for the actinomycosis fungus ("method No. 1").

FIG. 2.—Unusual type of development of the timothy hay bacillus in the lung of rabbit. Tissue stained with Ehrlich's acid hæmatoxylin; mycelia stained by the safranin method of Babes.

FIG. 3.—Actinomyces development of bacillus tuberculosis. Tubercle in lung of rabbit thirty days after intravenous injection of pure culture of bacillus tuberculosis. Partly decolorized with acid alcohol after staining in carbol-fuchsin.

FIG. 4.—Actinomyces type of development of butter bacillus Rabinowitsch. Kidney of rabbit after intravenous injection of pure culture. Staining as per Fig. 2.

FIG. 5.—Actinomyces type of development of timothy hay bacillus in lung of rabbit after intravenous injection of pure culture. Staining as per Fig. 2.

